Nitroglycerine and sodium trioxodinitrate: from the discovery to the preconditioning effect.

UNIVERSITÀ DEGLI STUDI DI TORINO

Nitroglycerine and Sodium Trioxodinitrate: From the Discovery to the Preconditioning Effect

Pasquale Pagliaro*, Donatella Gattullo and Claudia Penna

Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Italy

*Address for the correspondence:
Dr Pasquale Pagliaro
Dipartimento di Scienze Cliniche e
Biologiche Università di Torino
Ospedale S. Luigi, Regione Gonzole,10
10043 ORBASSANO (TO) Italy
Tel: 39-11 6705430/5450
fax: 39-11 9038639
e-mail: pasquale.pagliaro@unito.it

Abstract

The history began in the 19th century with Ascanio Sobrero (1812-1888), the discoverer of glycerol trinitrate (nitroglycerine, NTG), and with Angelo Angeli (1864-1931), the discoverer of sodium trioxodinitrate (Angeli's salt). It is likely that Angeli and Sobrero have never met, but their two histories will join each other more than a century later. In fact, it has been discovered that both NTG and Angeli's salt are able to induce a preconditioning effect. Since NTG has a long history as an antianginal drug its *new discovered* property as a preconditioning agent has been also tested in humans. Angeli's salt properties as preconditioning and inotropic agent have been tested in animals only so far.

Key words: Angeli'salt; Nitroglycerine; Nitric oxide; Nitroxyl; Cardioprotection.

The professor of chemistry Alfonso Cossa (1833-1902) was the successor of Ascanio Sobrero at the University of Torino, Italy. Angelo Angeli was one of the best pupils of Cossa and Cannizzaro. The latter was somehow involved in the story that saw Sobrero against Piria in a series of battles for university chair assignments around 1855. At the end, Piria settled in Torino and Cannizzaro in Genoa, against the wishes of Sobrero. However, in all this flurry of fights between academics there are not records of a meeting between Angeli and Sobrero.

In this short narrative review, we will see that the history has begun in the 19th century with Ascanio Sobrero and with Angelo Angeli, who discovered *nitroglycerine* and *sodium trioxodinitrate*, respectively, and we will see that the two histories will join in the 20th century with the discovery of some common physiological mechanisms of the two compounds (Fig. 1).

Nitroglycerine (NTG) was discovered in 1847 by Sobrero working at the University of Torino. Sobrero was born in Casale Monferrato (Alessandria, Piedmont) on the12th October 1812. He had gotten the degree in Medicine at the “Università di Torino” in 1833. His interest for chemistry was due to the uncle, the general Carlo Sobrero. Ascanio Sobrero worked as an assistant to professor Pelouze in Paris and then in 1845 became professor of chemistry in Torino[1]. He initially considered nitroglycerine to be far too dangerous to be of any practical use. Sobrero is quoted to have said: "*When I think of all the victims killed during nitroglycerine explosions, and the terrible devastation that has been committed, I am almost ashamed to admit to be its discoverer. The worst is that these crimes will continue to occur in the future…. because of the wickedness of human soul".* Another of Pelouze's pupils was the young Alfred Nobel, who took the knowledge back to the “Nobel family's defunct armaments factory”, and began experimenting with the compound around 1860. Although Nobel always acknowledged and honoured Sobrero as the man who had discovered nitroglycerine, Sobrero was dismayed both by the uses to which the terrible power of the explosive had been put, and by the fame and fortune accorded to Nobel because of it. He felt he had been subject to an injustice. Sobrero also discovered the photochemical oxidation of hydrate of trementine of

pinol, the so called "*Sobrerolo",* which is called in that way in his honour. Sobrerolo is still used as respiratory stimulant. He was a member of the "Accademia delle Scienze" of Torino. He died in Torino on 26th December 1888.

History does not record why NTG was first applied to the relief of human suffering. Sobrero tasted the compound and found that *"a very minute quantity upon the tongue produces a violent headache"*. It fell, however, to Guthrie in 1859 to have the first note on the fact that *nitrite of amyl* caused flushing of the face. Guthrie proposed that the compound could be used as a resuscitative[1,2]. However, it was Lauder Brunton[3] to first use nitrite of amyl for the relief of angina pectoris in 1867. Brunton wrote: *"to my delight the experiment proved a complete success… the patient's face became flushed, the pulse, instead of being small and thready, became full and bounding, and the (anginal) pain almost instantaneously disappeared"* [3]. Brunton also noted that NTG had an action very similar to amyl nitrite but he hesitated to give it to patients as he *"used to get such an awful headache from working with it"*[3], an obvious allusion to cerebral vasodilatation and increase in cerebral blood flow.

After Brunton had first recommended nitrate therapy against angina in the *Lancet*[3] the efficacy of nitrates in coronary artery disease has been well established. There is no doubt on this: NTG remains the treatment of choice for relieving angina[1,4-9].

More recently, apart from preload reduction and coronary vasodilatation, a number of beneficial effects of NTG and other nitric oxide (NO) donors have emerged, such as improved endothelial function[4-6], inhibition of platelet aggregation and cyclic flow variations[7], improvement of the efficiency of energy use[8], and preservation of contractile function during myocardial ischemia/reperfusion (I/R)[9,10].

However, despite the large body of research in this field and a wide clinical use of nitrates, it is noteworthy that about 165 years after Sobrero first synthesized nitroglycerine, we remain with at least four questions of fundamental importance: *1)* How do nitrates work? *2)* Why do nitrates stop working upon continuous administration? *3)* Do nitrates induce a long-term effect? and *4)* What are the long-term effects of nitrate

therapy on clinical outcome? Interestingly, these questions, which appear to be closely interrelated, are still without definitive answers.

In this report we will not try to definitely answer to these issues, but analyze some studies related with these questions. We will mainly consider those studies that have shown a “new mechanism” of NTG as trigger of cardioprotection (*i.e.* preconditioning effects), as this particular effect represents the *"trait d'union"* with the discovery of Angeli’s salt by Angelo Angeli. In particular we will consider how about 30 years ago a new interest on these compounds restarted.

Ignarro et al.[11] apparently answered the first of the above posed questions. They hypothesized that NO release mediates the effects of nitrates. This is true, but about 60% of the arterial vasodilatatory effect of nitroglycerine is, at least *in vitro*, attributed to the opening of potassium channels in endothelial cells[5,6,12]. Nitric oxide itself may act as potassium channel opener[13]. Recent observations also suggest that under certain circumstances nitroglycerine might not act through NO release[14].

Nevertheless, following the discovery of *endothelium derived relaxing factor* (EDRF)[15], and its identification as NO[16,17] there was a revival of interest on nitrite-containing compounds. Afterward thousands of papers have been published on these vasodilator compounds. It is now clear that NO may be produced by constitutive enzymes, namely endothelial NO synthase (eNOS) and neuronal NOS (nNOS), and inducible enzyme (iNOS), as well as by non-enzymatic processes[18-22].

The importance of NO in biology has been highlighted in 1992 by the Science magazine which defined nitric oxide the molecule of the year, and in 1998 when Furchgott, Ignarro and Murad were awarded with the *Nobel Prize in ‘‘Physiology or Medicine’’* for their pioneering research in this field.

After about 160 years from its discovery, studies have identified *"long-term"/"new mechanism"* of action for NTG in animals[13,19] and humans[10,23-25]. The works of Pagliaro and co-workers[13,24,26] on preconditioning have been performed at the University of Torino: "*history repeats itself*".

To better understand this relatively "new mechanism" we should spend some words on the definition of preconditioning (PC). The stimulus to induce PC consists in a series of brief (a few minutes) ischemia and reperfusion. Originally described as an immediate adaptation of the heart to brief sub lethal ischemia[27], it is now recognized that ischemic PC consists of two distinct phases: an early phase and a late phase of protection, which can be also induced pharmacologically. The early phase occurs immediately after the PC stimulus and induces robust protection. The early PC is short lasting (1 to 3 hours). This short period of efficacy limits its clinical relevance. In contrast, the late phase of PC develops 12 to 24 hours after the initial stimulus and lasts 3 to 4 days[13,19.] Unlike the early phase, the late phase of ischemic PC protects not only against myocardial infarction but also against myocardial stunning[13,19]. The late phase of PC is particularly interesting because it provides a long lasting and robust protection. Also angina can induce protection against myocardial infarction in adult, but this possibility is lost in elderly patients[28,29].

Besides the short period of ischemia, several other stimuli, including NO donors, may trigger PC. Because of this fact a considerable interest has been focused on PC and its clinical exploitation. In particular, the possibility to induce pharmacological PC makes this phenomenon really interesting from the viewpoint of translational medicine from bench to bedside, especially in the case of programmed intervention which can jeopardize the heart. The interrelation between ischemic preconditioning, NTG and aging heart has been explored and demonstrated by several groups, in animals and human studies[28,30-41]. In preconditioning a pivotal role is played by reactive oxygen species (ROS) production by mitochondria and NO formation by enzymatic (NOS) and non-enzymatic origin, which participate to the cardioprotection in the early stage and to the induction of enzyme expression, such as iNOS, cyclooxygenase (COX)-2 and superoxide dismutase (SOD), in the late phase of protection. In this context, particularly important is the role of mitochondrial permeability transition pore (mPTP). It seems that mPTP opening is a two-edged sword, with both protective (transient opening) and deleterious (prolonged opening) actions in both the pre- and

postischemic phases[22,42]. In particular, it has been suggested that a transient mPTP opening mediates preconditioning-induced protection *via* formation of small bursts of ROS[43] .. Importantly, it has been observed in humans that NTG also causes preconditioning-like improvement of flow-mediated dilation *via* ROS production and mPTP opening[44].

The role of mitochondria, ROS signaling, and NO from eNOS and iNOS in the early and late NTG preconditioning has been studied by several groups (the reader is kindly redirected to extensive reviews on this topic; *e.g,*[5,13,19,21,22,30,37,45]).

Of note, GRACE investigators have shown using data from 52.693 patients that chronic nitrate therapy is associated with better presentation and evolution of acute coronary syndromes[32]. This observation might represent an answer to the above questions marked with number 3 and 4.

Recent, interesting experimental studies that support beneficial effects of chronic NO bioavailability are those in which Bolli and coworkers performed either the gene transfer of iNOS[46] or the cardiomyocyte-restricted overexpression of extracellular SOD (ecSOD)[47], in mice. With iNOS gene transfer they observed long-term (1 year) cardioprotection against I/R injury, without negative functional consequences[42]. With cardiac-specific ecSOD overexpression they observed the attenuation of the levels of ROS by increased NO availability in response to I/R and the protection against reperfusion injury[47]. However caution must be used, in fact, for instance, iNOS expression in peripheral blood cells may mediate myocardial I/R injury[48]. These studies pave the way for further pre-clinical testing of gene therapy.

The role of NO as a trigger and mediator of PC is not entirely clear. Although it is not clear whether or not endogenous NO is less important in early preconditioning's protection against myocardial infarction[9,39,49], exogenous NO triggers early PC mainly through a free radical mechanism[26,39]. Yet, nitric oxide acts as a trigger and mediator in delayed preconditioning's protection against both myocardial infarction and stunning[13,19,26]. Importantly, nitroglycerine induces delayed preconditioning against myocardial stunning through a protein kinase C–dependent pathway[40,50]. The study of Banerjee et al.[40] and other studies on the role of NO in ischemic preconditioning have recently confirmed in animals and humans the possibility to

induce late PC with NTG[23,24]. The ability of NO-releasing agents such as nitrates to mimic the late phase of ischemic PC supports the possibility of *novel clinical applications* of these drugs. We demonstrated that NTG-induced preconditioning can positively affect hemodynamics during subsequent exercise in patients suffering from stable angina even 48 hours later from NTG administration. In fact, we found that heart rate, stroke volume, myocardial contractility and cardiac output at peak exercise were improved during the late PC period induced by the transdermal administration of NTG[24]. Importantly, a late PC-mimetic effect that improved exercise capacity during exercise and mitigated the ECG manifestations of ischemia was also observed 48 hours after the ischemia elicited by an exercise test[23]. To the best of our knowledge Crisafulli et al.[24] were the first that analyzed global performance in the same subjects in which was measured ECG during the late PC period induced by NTG or exercise. A positive effect of tonically released NO on cardiac function in healthy humans has also been suggested by Rassaf et al.[51]. These authors reported that inhibition of endogenous NO release reduced, whereas restoration with a NO donor, S-nitrosoglutathione, increased heart function.

The precise mechanism by which NTG and NO protect against ischemia remains to be elucidated, but it appears to involve the activation of soluble guanylate cyclase (sGC), given that both the alleviation of stunning and the reduction in infarct size are abrogated by the selective sGC inhibitor ODQ. It has been also suggested that NO protects by up regulating and activating COX-2[9,41]. The opening of K_{ATP} channels by NTG[12,13] can also play a role in its capacity to induce cardioprotection. S-Nitrosylation of proteins may play also a pivotal role in cardioprotection[21,22,45]. In fact, much of NO-triggered signaling appears to result from S-Nitrosylation, including the regulation of vascular sGC[52]. The role for S-Nitrosylation in altering protein localization has not been well studied in heart. However, S-Nitrosylation could protect by influencing the activity of mitochondrial proteins and other cell organelles. It has also been shown in other cell types to alter the localization of proteins and thereby altering cell death signaling. S-Nitrosylation can also protect by shielding thiol groups from oxidation and thereby allowing more rapid recovery of protein function in post-ischemic phase. Whether S-Nitrosylation mediates protection by the sum of these multiple pathways

or whether S-Nitrosylation of one or two proteins is of primary importance in mediating cardioprotection is unclear at this time[21,22].

The prognosis of long-term nitrate therapy is complicated by the tolerance phenomenon and the potential induction of endothelial dysfunction mainly by ROS-stress, which may have negative prognostic implications. Already Brunton noted that if NTG was used for a long time as relief of angina pain "…*the dose requires to be increased before the effect is produced*…", for instance the first reference to what we now refer to as nitrate tolerance[3]. The nitrate tolerance is also connected with the well known phenomena of *Monday disease* and nitrate-withdrawal/overcompensation by *Sunday Heart Attacks* observed in workers in the explosives industry, in the nineties. The history of these phenomena is elegantly reported in the review of Marsh and Marsh[1].

In the long run efficacy of nitrates in most patients is potentially limited both by *de novo* vascular and platelet hypo-responsiveness (referred to as *nitrate resistance*) and by the potential development, during chronic nitrate therapy, of an attenuation of such responses, termed *true nitrate tolerance* and *pseudo-tolerance* (see Münzel et al.[54] for a review). Many studies in the near past considered the tolerance phenomenon of NTG in an attempt to respond to the above question marked with number two (Why do nitrates stop working upon continuous administration?). Some recent studies, including those of Münzel & Gori and coworkers, started to solve this complicated issue[53-57]. These studies suggest new mechanisms to explain nitrate tolerance, provide evidence for different potencies among nitrates to produce tolerance and demonstrate in clinical investigations that tolerance might be reduced by addition of *hydralazine* or *folic acid*[54-57]. Beneficial effects in attenuating nitrate tolerance are also observed with therapy with the angiotensin-AT1 receptor blocker *telmisartan* and the statin *atorvastatin*, which also prevents nitroglycerin-induced endothelial dysfunction in healthy humans[56,57]. In particular, it has been suggested that a role is played by reactive oxygen species and protein kinase C activation in developing nitrate tolerance[54]. Also adverse phosphorylation and S-Glutathionylation of endothelial nitric oxide synthase have been involved in tolerance development[58,59].

Of note, also the protective effect of acute NTG may be attenuated by daily administration of this drug. However, pentaerythrityl tetranitrate (PETN), which is an organic nitrate with intrinsic antioxidant properties, may induce preconditioning-like effects against post-ischemic endothelial dysfunction, which are maintained even after PETN prolonged administration in humans. Such a difference has been attributed to differential effects of the two compounds on the activity of mitochondrial aldehyde dehydrogenase, an enzyme also involved in ischemic- and nitrate-induced preconditioning[60,61].

In addition, a variety of studies have suggested that nitrates are endowed with antiplatelet, antioxidative, antiadhesive and antiproliferative effects. These effects might also be useful in coronary artery disease (CAD) patients as *in vivo* animal experiments provided evidence that therapy with different nitrates could reduce the progression of atherosclerosis and endothelial dysfunction[6,58]. Finally, a large clinical trial with the potassium channel opener and nitrovasodilator, *nicorandil*, has shown beneficial effects in CAD patients, who had been taking oral nicorandil during the followup[59].

Thanks to *preconditioning*, the history of NTG, which started with Sobrero, has been joined to the history of so-called Angeli's salt ($Na_2N_2O_3$; sodium trioxodinitrate), which was originally synthesized in the late 1800s by Angelo Angeli, another Italian chemist[62,63].

Angeli was born in Tarcento (Udine), on 20th August 1864. Since when he was young he demonstrated a remarkable interest for chemistry also encouraged by his uncle Giovanni Carnelutti (1850-1901), pupil of Cannizzaro. Because of his bashful character Angeli did not attend meetings and did not talk in front of people. This may be one of the reasons why there is no news of a meeting between Angeli and Sobrero, even though Angeli was a member of the "Accademia Nazionale delle Scienze" as Sobrero. This shyness did not help Angeli to gain honour in the Italian academy. However, Willstaetter (1872-1942), Nobel Prize in 1915, wrote about Angeli: *"The Angeli's work is the best among those of Italian chemists both for originality and value"*. Angeli was the father of the so-called "*Theorie der Vernachlässigung des Benzolkerns*" (*i.e.* The

theory of the neglected benzoic nucleus). He also studied the relation between compounds structure and olfactory properties[57]. Angelo Angeli died on 31st May 1931.

Recently, Angeli's salt has regained the interest of biologists, although there are not yet human *in vivo* data for this substance. At present Angeli's salt is the only compound available that spontaneously releases nitroxyl (HNO, a nitric oxide sibling also known as nitrosyl hydride) under physiological conditions[64,65]. Clearly Angeli' salt is not a NO donor, but it is a HNO donor which has distinct effects. Sulfohydroxamic acid derivatives such as Piloty's acid also spontaneously release HNO, but only under basic conditions, and it undergoes rapid oxidation yielding NO rather than HNO[64,65]. It has been reported that HNO elicits vasorelaxation in both bovine intrapulmonary artery and the rabbit aorta by a sGC-dependent pathway[64,65]. It is now clear that the thiol-donating agent L-cysteine can discriminate the vasodilatative profile of HNO from that of NO or nitrosothiols: effects of HNO, donated by Angeli' salt, can be blocked by this agent, whereas the action of NO, donated by NO-donors, is potentiated[66].

Recently, a comparative study with Angeli's salt and DEA/NO (a pure NO-donor) determined that equimolar HNO released by Angeli's salt appeared to be a more effective preconditioning agent than NO[26]. In fact, post-ischemic (2 h) contractility was similarly improved with ischemic PC or pre-exposure to Angeli's salt, compared with control or DEA/NO-treated hearts. Infarct size and lactate dehydrogenase release were also significantly reduced in ischemic PC and Angeli's salt groups, whereas DEA/NO was less effective in limiting necrosis[26].

Recently, Paolocci and co-workers[67-72] have shown that the nitroxyl donor Angeli's salt appears to be very good candidate to treat failing hearts that are characterized by pressure overload, poor contractile function and delayed relaxation. In fact when it was administered to normal, conscious dogs and those with heart failure an enhancement of heart contractility and lusotropy were observed together a vasodilator action[63,67]. The complexity of the mechanisms of the beneficial cardiac effects of HNO has also been examined in cardiomyocytes. It seems that the main mechanism of action of HNO uses its *thiophylic nature*

as a vehicle to interact with redox targets such as cysteines, which are located in key components of the cardiac electromechanical machinery ruling myocardial function[70,71].

Calcitonin gene-related peptide (CGRP) does not account for direct HNO-evoked positive inotropy-lusitropy. However, HNO-induced CGRP release might play an indirect role in improving cardiac function and may, in part, explain HNO-induced vasodilation[72,73]. In the cardiovascular system, CGRP is a potent vasodilator and induces positive cardiac inotropy in several species including humans[74]. HNO is also an effective inhibitor of human platelet aggregation *in vitro*[75].

New nitroxyl donors not only would confirm that the physiological effects seen with Angeli's salt are truly due to HNO, but they also would help researchers determine if the rate of HNO release had any effect on the resulting physiological response. Of course, to propose HNO donors for the treatment of heart failure, they need to be tested in the long terms and in humans.

Concluding remarks

We owe a great debt to Sobrero, Angeli, Brunton and the 1998 Nobel laureates for their discoveries. Nowadays NTG is scarcely used as an explosive, but remains the drug of choice for angina relief and its new property as preconditioning agent make it a very interesting compound.

Nitrates are part of combined modality therapy for myocardial ischemia, chest pain, hypertension and heart failure in patients with acute coronary syndrome. Nevertheless, the use of NTG prolonged administration is steadily declining, and it is mainly administered in settings of refractory angina[61]. The use of new methods of administration of nitrates, such as atypical therapeutic schemes able to determine preconditioning-like effects and the association with antioxidants (*e.g.,* hydralazine) capable of modifying the tolerance and endothelial dysfunction might open new perspectives for reconsidering the use of NTG in the long term.

Angeli' salt with its inotropic and preconditioning properties has opened a new frontier for the research on this field. Of course, because of its clinical use, more is known about NTG than Angeli' salt, but for instance working with these two compounds which have similar and different features we can understand more

about their mechanisms of action and their usefulness. In fact, we must stress that despite about 165 years of clinical practice and research, nitrates remain intriguing drugs, whose mechanisms of action still need investigation. We hope the present article will be a stimulus for further discussions and research.

Acknowledgments and Funding/Support

The authors thank the INRC, Compagnia di San Paolo of Torino, the University of Torino and the Regione Piemonte for financial support to their research. We would like to thank Dr Barbara Mognetti for insightful suggestions.

References

1. Marsh N, Marsh A. A short history of nitroglycerine and nitric oxide in pharmacology and physiology. *Clin Exp Pharmacol Physiol* 2000;**27**:313-319.
2. Goodman LS, Gilman A. The Paharmacological Basis of Therapeutics, 1955; 2nd edition, p730, Macmillan: New York.
3. Brunton TL. Use of nitrite of amyl in angina pectoris. *Lancet* 1867;**ii**:97-98.
4. Drexler H. Nitric oxide and coronary endothelial dysfunction in humans. *Cardiovasc Res* 1999;**43**:572–579.
5. Luca MC, Liuni A, Muxel S, et al. Chronic pharmacological preconditioning against ischemia. *Clin Hemorheol Microcirc* 2011;**49**:287-293.
6. Lisi M, Oelze M, Dragoni S, et al. Chronic protection against ischemia and reperfusion-induced endothelial dysfunction during therapy with different organic nitrates. *Clin Res Cardiol* 2012;**101**:453-459.
7. Yao SK, Ober JC, Krishnaswami A, et al. Endogenous nitric oxide protects against platelet aggregation and cyclic flow variations in stenosed and endothelium-injured arteries. *Circulation* 1992;**86**:1302-1309.
8. Recchia FA, McConnell PI, Loke KE, et al. Nitric oxide controls cardiac substrate utilization in the conscious dog. *Cardiovasc Res* 1999;**44**:325-332.
9. Heusch G, Post H, Michel MC, et al. Endogenous nitric oxide and myocardial adaptation to ischemia. *Circ Res* 2000;21;**87**:146-152.
10. Schulz R, Kelm M, Heusch G. Nitric oxide in myocardial ischemia/reperfusion injury. *Cardiovasc Res* 2004;**61**:402-413.
11. Ignarro LJ, Lippton H, Edwards JC, et al. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. *J Pharmacol Exp Ther* 1981;**18**:739-749.
12. Gruhn N, Boesgaard S, Eiberg J, et al. Effects of large conductance Ca(2+)-activated K(+) channels on nitroglycerin-mediated vasorelaxation in humans. *Eur J Pharmacol* 2002;**446**:145-150.
13. Pagliaro P. Differential biological effects of products of nitric oxide (NO) synthase: it is not enough to say NO. *Life Sci* 2003;**73**:2137-2149.
14. Kleschyov AL, Oelze M, Daiber A, et al. Does nitric oxide mediate the vasodilator activity of nitroglycerin? *Circ Res* 2003;**93**:e104-112.
15. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;**288**:373-376.

16. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;**327**:524-526.

17. Furchgott RF. An historical survey and prospects of research on EDRF. *Nihon Heikatsukin Gakkai Zasshi* 1987;**23**:435-440.

18. Zweier JL, Samouilov A, Kuppusamy P. Non-enzymatic nitric oxide synthesis in biological systems. *Biochim Biophys Acta* 1999;**1411**:250-262.

19. Bolli R. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. *J Mol Cell Cardiol* 2001;**33**:1897-1918.

20. Martin C, Schulz R, Post H, et al. Microdialysis-based analysis of interstitial NO in situ: NO synthase-independent NO formation during myocardial ischemia. *Cardiovasc Res* 2007, **74**:46-55.

21. Pagliaro P, Moro F, Tullio F, Perrelli MG, Penna C. Cardioprotective pathways during reperfusion: focus on redox signaling and other modalities of cell signaling. *Antioxid Redox Signal* 2011;**14**:833-850.

22. Penna C, Perrelli MG, Pagliaro P. Mitochondrial pathways, permeability transition pore, and redox signaling in cardioprotection: therapeutic implications. *Antioxid Redox Signal* 2013;**18**:556-599.

23. Leesar MA, Stoddard MF, Dawn B, et al. Delayed preconditioning-mimetic action of nitroglycerin in patients undergoing coronary angioplasty. *Circulation* 2001;**103**:2935-2941.

24. Crisafulli A, Melis F, Tocco F, et al. Exercise-induced and nitroglycerin-induced myocardial preconditioning improves hemodynamics in patients with angina. *Am J Physiol Heart Circ Physiol* 2004;**287**:H235-H242.

25. Heusch G. Nitroglycerin and delayed preconditioning in humans: yet another new mechanism for an old drug? *Circulation* 2001;**103**:2876-2878.

26. Pagliaro P, Mancardi D, Rastaldo R, et al. Nitroxyl affords thiol-sensitive myocardial protective effects akin to early preconditioning. *Free Radic Biol Med* 2003;**34**:33-43.

27. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;**74**:1124-1136.

28. Abete P, Ferrara N, Cacciatore F, et al. Angina-induced protection against myocardial infarction in adult and elderly patients: a loss of preconditioning mechanism in the aging heart? *J Am Coll Cardiol* 1997;**30**:947-954.

29. Boengler K, Schulz R, Heusch G. Loss of cardioprotection with ageing. *Cardiovasc Res* 2009;**83**: 247-261.

30. Tomai F, Crea F, Chiariello L, Gioffrè PA. Ischemic preconditioning in humans: models, mediators, and clinical relevance. *Circulation* 1999;**100**:559-563.
31. Abete P, Cacciatore F, Testa G, et al. Ischemic preconditioning in the aging heart: from bench to bedside. *Ageing Res Rev* 2010;**9**:153-162.
32. Ambrosio G, Del Pinto M, Tritto I, et al. GRACE Investigators.Chronic nitrate therapy is associated with different presentation and evolution of acute coronary syndromes: insights from 52,693 patients in the Global Registry of Acute Coronary Events. *Eur Heart J* 2010;**31**:430-438.
33. Battipaglia I, Scalone G, Milo M, Di Franco A, Lanza GA, Crea F. Upper arm intermittent ischaemia reduces exercise-related increase of platelet reactivity in patients with obstructive coronary artery disease. *Heart* 2011;**97**:1298-1303.
34. Crisafulli A, Melis F, Tocco F, et al. Delayed preconditioning-mimetic actions of exercise or nitroglycerin do not affect haemodynamics and exercise performance in trained or sedentary individuals. *J Sports Sci* 2007;**25**:1393-401.
35. Niccoli G, Altamura L, Fabretti A, et al. Ethanol abolishes ischemic preconditioning in humans. *J Am Coll Cardiol* 2008;**51**:271-275.
36. Penna C, Bassino E, Alloatti G. Platelet activating factor: the good and the bad in the ischemic/reperfused heart. *Exp Biol Med* (Maywood) 2011;**236**:390-401.
37. Penna C, Mancardi D, Raimondo S, Geuna S, Pagliaro P. The paradigm of postconditioning to protect the heart. *J Cell Mol Med* 2008;**12**:435-458.
38. Penna C, Mancardi D, Rastaldo R, Pagliaro P. Cardioprotection: a radical view Free radicals in pre and postconditioning. *Biochim Biophys Acta* 2009;**1787**:781-793.
39. Nakano A, Liu GS, Heusch G, et al. Exogenous nitric oxide can trigger a preconditioned state through a free radical mechanism, but endogenous nitric oxide is not a trigger of classical ischemic preconditioning. *J Mol Cell Cardiol* 2000;**32**:1159–1167.
40. Banerjee S, Tang X-L, Qiu Y, et al. Nitroglycerin induces late preconditioning against myocardial stunning via a PKC-dependent pathway. *Am J Physiol* 1999;**277**:H2488–H2494.
41. Shinmura K, Xuan YT, Tang XL, et al. Inducible nitric oxide synthase modulates cyclooxygenase-2 activity in the heart of conscious rabbits during the late phase of ischemic preconditioning. *Circ Res* 2002;**90**:602-608.
42. Hausenloy DJ, Ong SB, Yellon DM. The mitochondrial permeability transition pore as a target for preconditioning and postconditioning. *Basic Res Cardiol* 2009;**104**:189-202.
43. Hausenloy D, Wynne A, Duchen M, Yellon D. Transient mitochondrial permeability transition pore opening mediates preconditioning-induced protection. *Circulation* 2004;**109**:1714–1717.

44. Gori T, Di Stolfo G, Sicuro S, et al. Nitroglycerin protects the endothelium from ischaemia and reperfusion: human mechanistic insight. *Br J Clin Pharmacol* 2007;**64**:145-150.

45. Heusch G, Boengler K, Schulz R. Cardioprotection: nitric oxide, protein kinases, and mitochondria. *Circulation* 2008;**118**:1915-1919.

46. Li Q, Guo Y, Wu WJ, et al. Gene transfer as a strategy to achieve permanent cardioprotection I: rAAV-mediated gene therapy with inducible nitric oxide synthase limits infarct size 1 year later without adverse functional consequences. *Basic Res Cardiol* 2011,106:1355-1366.

47. Obal D, Dai S, Keith R, et al. Cardiomyocyte-restricted overexpression of extracellular superoxide dismutase increases nitric oxide bioavailability and reduces infarct size after ischemia/reperfusion. *Basic Res Cardiol* 2012,**107**:305-318.

48. Guo Y, Sanganalmath SK, Wu W, et al. Identification of inducible nitric oxide synthase in peripheral blood cells as a mediator of myocardial ischemia/reperfusion injury. *Basic Res Cardiol* 2012;**107**:253-260.

49. Post H, Schulz R, Behrends M, et al. No involvement of endogenous nitric oxide in classical ischemic preconditioning in swine. *J Mol Cell Cardiol* 2000;**32**:725-733.

50. Bolli R, Dawn B, Tang XL, et al. The nitric oxide hypothesis of late preconditioning. *Basic Res Cardiol* 1998;**93**:325-338.

51. Rassaf T, Poll LW, Brouzos P, et al. Positive effects of nitric oxide on left ventricular function in humans. *Eur Heart J* 2006;**27**:1699-1705.

52. Oppermann M, Suvorava T, Freudenberger T, et al. Regulation of vascular guanylyl cyclase by endothelial nitric oxide-dependent posttranslational modification. *Basic Res Cardiol* 2011;**106**:539-549.

53. Münzel T, Daiber A, Gori T. Nitrate therapy: new aspects concerning molecular action and tolerance. *Circulation* 2011;**123**:2132–2144.

54. Münzel T, Harrison DG. Evidence for a role of oxygen-derived free radicals and protein kinase C in nitrate tolerance. *J Mol Med* 1997;**75**:891-900.

55. Warnholtz A, Tsilimingas N, Wendt M, Münzel T. Mechanisms underlying nitrate-induced endothelial dysfunction: insight from experimental and clinical studies. *Heart Fail Rev* 2002;**7**:335-345.

56. Daiber A, Münzel T, Gori T. Organic nitrates and nitrate tolerance--state of the art and future developments. *Adv Pharmacol* 2010;**60**:177-227.

57. Liuni A, Luca MC, Di Stolfo G, et al. Coadministration of atorvastatin prevents nitroglycerin-induced endothelial dysfunction and nitrate tolerance in healthy humans. *J Am Coll Cardiol* 2011;**57**:93-98.

58. Knorr M, Hausding M, Kröller-Schuhmacher S, et al. Nitroglycerin-induced endothelial dysfunction and tolerance involve adverse phosphorylation and S-Glutathionylation of endothelial nitric oxide synthase: beneficial effects of therapy with the AT1 receptor blocker telmisartan. *Arterioscler Thromb Vasc Biol* 2011;**31**:2223-2231.

59. Kitakaze M, Asakura M, Kim J, et al. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet* 2007,**370**:1483-1493.

60. Lisi M, Oelze M, Dragoni S, et al. Chronic protection against ischemia and reperfusion-induced endothelial dysfunction during therapy with different organic nitrates. *Clin Res Cardil* 2012;**101**:453-459.

61. Münzel T, Gori T. Nitrate therapy and nitrate tolerance in patients with coronary artery disease. *Curr Opin Pharmacol*. 2013; doi:pii:S1471-4892(13)00004-0. 10.1016/j.coph.2012.12.008.

62. Angeli A. *Gazz Chim Ital* 1896;**26**:17.

63. Ferroni E. "Le Scienze Chimiche" in "Storia dell'Ateneo Fiorentino", 1989. Ed. F. & F. Parretti Grafiche, Firenze, pg. 625

64. Wink DA, Miranda KM, Katori T, et al. Orthogonal properties of the redox siblings nitroxyl and nitric oxide in the cardiovascular system: a novel redox paradigm. *Am J Physiol Heart Circ Physiol* 2003;**285**:H2264-H2276.

65. Fukuto JM, Chiang K, Hszieh R, Wong P, Chaudhuri G. The pharmacological activity of nitroxyl: a potent vasodilator with activity similar to nitric oxide and/or endothelium-derived relaxing factor. *J Pharmacol Exp Ther* 1992;**263**:546–551.

66. Pino RZ, Feelisch M. Bioassay discrimination between nitric oxide (NO.) and nitroxyl (NO-) using L-cysteine. *Biochem Biophys Res Commun* 1994;**201**:54–62.

67. Paolocci N, Katori T, Champion HC, et al. Positive inotropic and lusitropic effects of HNO/NO- in failing hearts: independence from beta-adrenergic signaling. *Proc Natl Acad Sci USA* 2003;**100**:5537-5542.

68. Gao WD, Murray CI, Tian Y, et al. Nitroxyl(HNO)-mediated disulfide bond formation between cardiac myofilament cysteines enhances contractile function. *Circ Res* 2012;**111**:1002-1011.

69. Ding W, Li Z, Shen X, et al. Reversal of isoflurane-induced depression of myocardial contraction by nitroxyl via myofilament sensitization to Ca^{2+}. *J Pharmacol Exp Ther* 2011;**339**:825-831.

70. Tocchetti CG, Stanley BA, Murray CI, et al. Playing with cardiac "redox switches": the "HNO way" to modulate cardiac function. *Antioxid Redox Signal* 2011;**14**:1687-1698.

71. Paolocci N, Wink DA. The shy Angeli and his elusive creature: the HNO route to vasodilation. *Am J Physiol Heart Circ Physiol* 2009;**296**:H1217-H1220.

72. Paolocci N, Saavedra WF, Miranda KM, et al. Nitroxyl anion exerts redox-sensitive positive cardiac inotropy in vivo by calcitonin gene-related peptide signaling. *Proc Natl Acad Sci U S A* 2011;**98**:10463-10468.

73. Favaloro JL, Kemp-Harper BK. The nitroxyl anion (HNO) is a potent dilator of rat coronary vasculature. *Cardiovasc Res* 2007;**73**:587-596.

74. Bell D, McDermott BJ. Calcitonin gene-related peptide in the cardiovascular system: characterization of receptor populations and their (patho)physiological significance. *Pharmacol Rev* 1996;**48**:253-288.

75. Bermejo E, Saenz DA, Alberto F, Rosenstein RE, Bari SE, Lazzari MA. Effect of nitroxyl on human platelets function. *Thromb Haemost* 2005;**94**:578-584.

FIGURE LEGEND

Figure 1. Time line shows the chain of some of the researches that led to an understanding of nitroglycerine and Angeli'salt as cardiotropic agents. EDRF= endothelium-derived relaxing factor.